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Case Report:

An acute outbreak of equine dysautonomia (equine grass sickness) in a group of 8 Przewalski's horses (*Equus ferus (caballus) przewalskii*)

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Abstract

Equine grass sickness (EGS) is a neurodegenerative disease affecting grazing equids, of which a single case of the chronic clinical presentation has previously been reported in a Przewalski's horse (*Equus ferus (caballus) przewalskii*). A group of 8 Przewalski's horses were moved to a new enclosure, recently vacated by a group of 4 Eastern kiang (*Equus kiang holdereri*) that showed no evidence of disease. After 23 days the first Przewalski horse showed clinical signs of acute EGS including flank sweating, belly kicking, rapid loss of body condition, cessation of faecal passage, nasogastric reflux and mouthing water. It was humanely euthanased within 48 hours due to lack of therapeutic response. Within 24 hours of this first case developing clinical signs, a further 5 Przewalski's horses showed similar clinical signs of acute EGS and were euthanased. All animals were confirmed acute EGS on post mortem demonstrating typical chromatolysis, cytoplasmic hypereosinophilia, cellular swelling, vacuolation, pyknosis and loss of nuclei in approximately 90% of neurones in the cranial cervical and cranial mesenteric ganglia and myenteric and submucosal plexi of the ileum. Two Przewalski's horses within the group showed no clinical signs of disease. No single pathogen was identified as the causal agent, but the epidemiological pattern of the outbreak was typical for that previously reported for acute EGS in domestic equids. All affected animals had a low antibody titre to *Clostridium botulinum* type C toxin, but so did the two surviving horses. This is the first report of acute EGS in a herd of Przewalski's horses.

Introduction

Equine grass sickness (EGS), also known as equine dysautonomia, is a disease seen in horses, ponies, donkeys and zebra in the UK, northern Europe (Hahn et al., 2005) and the southern parts of South America (Uzal et al., 1992). Similar dysautonomic syndromes have

been reported in other hind-gut fermenters such as Brown hares and domestic rabbits resulting in large bowel impaction and inability to swallow (Whitwell 1991; Whitwell and Needham 1996). No single causal agent has been definitively identified. A single case of chronic EGS has been reported in a Przewalski's horse and one in a zebra (Ashton et al., 1977). This report describes an outbreak of acute EGS in a group of eight Przewalski's horses (*Equus ferus (caballus) przewalskii*) recently moved to new pasture, of which six, aged from 3 to 8 years, developed typical clinical signs, were euthanased and the diagnosis confirmed on *post mortem* histopathological examination. Two co-grazing individuals, aged 11 and 22 years, were unaffected. A group of four Eastern kiang (*Equus kiang holdereri*) which grazed the same pasture immediately before, were unaffected. This is the first report of acute EGS in Przewalski's horses.

Case history and findings

In May 2010, an established group of eight female Przewalski's horses (*Equus ferus (caballus) przewalskii*) were moved to a new paddock within a wildlife park, which had previously been occupied by a group of four Eastern kiang (*Equus kiang holdereri*) for one year. This paddock had prior to that contained Przewalski's horses (including some of this study) for the period 1972-2007. None had received any medication in the preceding two months. Twenty-three days after the change of pasture, one Przewalski's horse developed clinical signs including: dullness; salivation from the corner of the mouth, followed by mouthing water in the pond in the enclosure; flank sweating; complete cessation of defaecation and rapid loss of body condition. A clinical examination prior to euthanasia demonstrated signs of colic, including flank watching and belly kicking as well as other clinical signs including sweating inappetance and no gastrointestinal sounds on auscultation. Further clinical signs included passive nasal reflux of stomach contents and yellow mucus in

the rectum consistent with acute EGS. The horse was humanely euthanased after 48 hours of first demonstrating clinical signs, due to worsening clinical signs of colic and lack of response to non-steroidal anti-inflammatory (meloxicam Metacam 20mg/ml® Boehringer Ingelheim at 0.6mg/kg intravenously) therapy. Within 24 hours of the clinical signs of the first case, five of the remaining seven horses developed similar clinical signs. Their deterioration was rapid over the ensuing 24-48 hours and all demonstrated the same clinical signs as the index case. Euthanasia on humane grounds was carried out on all of these additional cases. Clinical signs in all cases were consistent with acute EGS as described in domestic horses and included: depressed demeanour; anorexia; salivation from the corners of the mouth; mouthing/playing with water; ptosis; flank watching; belly kicking; patchy sweating; muscle tremors/twitches in the forelegs; elevated heart rates (ranging from 60-90 beats per minute); absence of gut sounds on auscultation; passive green nasal reflux and rectal examination demonstrating no faeces but yellow mucus in the rectum and distended small intestine (Pirie et al. 2014). Ages of the six affected horses were three years (n=2), four years (n=3) and eight years (n=1). Two co-grazing Przewalski's horses, aged 11 and 22 years, were unaffected and remain healthy. These two unaffected Przewalski's horses were removed from the pasture at the time of the euthanasia of the affected cases.

Paddock soil/sward sampling

Soil samples were collected from the paddock and subjected to a variety of microbial culture techniques. These included *Clostridium botulinum* isolation agar® used according to manufacturer's guidelines (Sigma-Aldrich, Dorset UK) which revealed a moderate growth of *Clostridium botulinum*, and *Clostridium perfringens* agar base® used according to manufacturer's guidelines (Acumedia Neogen Europe, Ayr UK) which revealed a moderate

growth of *Clostridium perfringens*. It also included culture on Sabouraud's and malachite green agar at 37°C (Castella et al., 1997) which yielded a moderate growth of *Fusarium nivale*. There was no evidence of plants that have been previously investigated in connection with a possible link to EGS in the paddock such as alsike clover, although *Ranunculus* spp. plants were seen. The soil had low levels of organic nitrogen (21.6-25.2mg/L (normal laboratory mean of livestock grassland in Scotland 40mg/L and range 15-60mg/L); measured by colorimetry) and low nitrate (13.32-16.56mg/L (normal laboratory mean of livestock grassland in Scotland 20mg/L and range 8-30mg/L); measured by colorimetry) with a low organic nitrogen: nitrate ratio (1.52-1.62 (normal laboratory mean of livestock grassland in Scotland 2 and range 0.5-5)) and a pH of 4.7-4.9 (measured by electrometry: SOP 2001).

Post mortem findings

Gross *post mortem* examination of all affected horses showed evidence of previous passive reflux of gastric ingesta into the oesophagus, and an apparently flaccid, distended gastrointestinal tract with impacted faeces in the colon and mucus in the rectum. Histopathological examination confirmed chromatolysis, cytoplasmic hypereosinophilia, cellular swelling, vacuolation, pyknosis and loss of nuclei in approximately 90% of neurones in the cranial cervical and cranial mesenteric ganglia and in the myenteric and submucosal plexi of the ileum confirming EGS. No evidence of any significant concurrent disease was present. Serological testing for *Clostridium botulinum* type C, using a direct ELISA adapted from the method described by Poxton et al. (1997), demonstrated low antibody titres in all six affected animals and the two which survived.

Of the six animals, *Clostridium botulinum* was cultured from the large intestine of one and *Clostridium perfringens* from two using the techniques described above, but *Fusarium* spp. were not isolated using Sabouraud's or malachite green agar culture media at either 27 or

37°C. Visual assessment of gastrointestinal contents showed no obvious plants known to be poisonous to equids, although it is acknowledged that the process of mastication and digestion complicates this.

Discussion

This cases series represents the first reported outbreak of acute EGS in Przewalski's horses. A single case of chronic EGS has previously been reported in a Przewalski's horse and one in a zebra (Ashton et al., 1977); another atypical case report in a young zebra has also been published (Wales et al., 2001). The prevalence on premises with a previous history of EGS is reported to be between 0.4-16% (Doxey et al., 1991). No single causal agent has been definitively identified, although a number of suggestions have been made including *Clostridium botulinum* type C, *Clostridium perfringens* (particularly type A), *Fusarium* spp., alsike clover (*Trifolium hybridum*) and *Ranunculus* spp. toxin (Hunter et al., 1999; McCarthy et al., 2001; Waggett et al., 2010; Michl et al., 2011). Acute EGS is thought to be due to ingestion of a neurotoxic agent causing extensive damage to autonomic neurons (Hunter et al., 1999; Shotton et al., 2011). In this outbreak, no one consistent toxin producing organism that has previously been suggested as a causal agent was recovered from all six affected animals and no previous case of EGS had been recorded in the last 40 years on this site. No evidence of a serological immune response was present in any of the animals affected or either of the two that survived the outbreak to *Clostridium botulinum* type C toxin. All clinically affected Przewalski's horses in this outbreak demonstrated clinical signs typical of acute EGS as described in domestic equids (Pirie et al. 2014).

Other epidemiological factors reported to be associated with increased risk of EGS include a recent move to a different pasture/change of food type, breed, age, (most between 2-12 years with those aged 3-5 years six times more likely to contract EGS), time of year

(late spring/early summer), concentration of organic nitrogen and acidity of the soil (high organic nitrogen and loam/acidic soils having a higher risk), and frequent anthelmintic administration or repeated use of ivermectin (Doxey et al., 1991; McCarthy et al., 2001; Newton et al., 2004; McCarthy et al., 2004; French et al., 2005). In this outbreak, the horses had moved pasture 23 days previously. Przewalski's horses have not been included in the study population of any epidemiological studies, therefore there is no available information regarding the species risk of EGS in comparison to other equids. The age of those affected was typical of EGS with the two surviving animals being the two oldest animals. The cases occurred in May after a period of warm wet weather. However, the soil had a low concentration of organic nitrogen which is not typical of pastures in which EGS most frequently occurs, although the soil pH was acidic which is consistent with previous studies (Newton et al., 2004; McCarthy et al., 2001). In addition, no horse had been treated with anthelmintics such as ivermectin within two months of the outbreak. The group of four Eastern kiang, that grazed the same field for a period of one year immediately prior to the Przewalski's horses, showed no clinical signs and remained healthy despite being a potentially susceptible equid, within the age range at highest risk. However, this may not be unexpected since they are asses rather than horses, and domestic donkeys and mules appear more resistant to EGS than domestic horses (McCarthy et al., 2001).

This study suggests that Przewalski's horses are susceptible to acute EGS, with typical clinical and pathological changes, and that Eastern kiang may be less susceptible. Although the epidemiology of the outbreak is similar to many previous reports on EGS in domestic equids, it still does not identify the likely causal agent(s). EGS should be considered as a differential diagnosis for any acute onset colic episode in Przewalski's horses.

Conflict of Interest Statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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